Chapter 3

Synthesis of Pyrazolines as Antimicrobials, Antioxidants and Anti-inflammatory agents
CHAPTER 3. Synthesis of Pyrazolines as Antimicrobials, Antioxidants and Anti-inflammatory agents

3.1. Motivation for the current work

Heterocyclic compounds find a prime place in the medicinal chemistry as most of the synthetic drugs as well as naturally occurring biologically important compounds possess at least one heterocyclic ring. Five-membered heterocycles with a conserved vicinal diaryl substitution pattern are ideal representatives of a recurring core structure that is found in numerous biologically active compounds, including cyclooxygenase inhibitors, antioxidants, kinase inhibitors, GPCR antagonists and agonists, phosphatase inhibitors and dopamine transporter inhibitors.\(^1\)

Pyrazolines are prominent nitrogen containing five-membered heterocyclic compounds possessing broad spectrum of biological activities like antimicrobial,\(^2\)-\(^4\) anti-inflammatory,\(^5\)-\(^9\) LDL-oxidation inhibitors,\(^10\) etc. 1,3,5-Trisubstituted pyrazolines occupy a unique position amongst a large array of medicinally important pyrazoline derivatives and their evaluation as anti-inflammatory, antimicrobial as well as antioxidant agents has attracted much attention in the recent past.\(^11\),\(^12\) Antioxidants are believed to counteract the harmful effects of reactive oxygen species (ROS) and regulate the physiological defence systems and therefore could be useful for prevention or treatment of oxidative stress-related diseases.\(^13\),\(^14\) Oxidative stress is implicated in the pathogenesis of several human diseases such as cancer, aging, atherosclerosis, neurodegenerative diseases etc.,\(^15\)-\(^17\) by way of inducing damage to proteins, lipids, DNA etc. ROS is a term collectively used for a variety of oxygen derived molecules which oxidize most of the biomolecules. ROS include radical, ionic as well as neutral species such as superoxide anions (O\(_2^−\)), hydroxyl radicals (OH\(^−\)), nitric oxide (NO), hydrogen peroxide (H\(_2\)O\(_2\)), hypochlorous acid (HOCl), peroxynitrite (ONOO\(^−\)) etc. Considerable research efforts have been devoted in the recent past to evaluate the phenolic derivatives as antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), butylated hydroxyquinone (TBHQ), flavonoids, tannins, phenolic acids etc., all of which possess good antioxidant activity.
The prescription of co-administration of multiple drugs for treatment of any particular disorder such as inflammatory conditions associated with some microbial infections may inflict added health problems especially in patients with impaired liver or kidney functions. A mono therapy of an AI drug with antimicrobial as well as antioxidant properties will be better from the pharmaco-economic point as this will enhance patient compliance and in case that this AI-antimicrobial and antioxidant agent shows minimum adverse effects and high safety margin, this drug will be highly desirable. Motivated by aforementioned findings and to broaden the scope of our ongoing research on developing novel heterocyclic compounds of potential medicinal interest,\textsuperscript{18-23} we synthesized and investigated the AI as well as antimicrobial activity of a series of 1,3,5-triarylsubstituted pyrazoline derivatives. Additionally, we evaluated potential of selected 1,3,5-triarylsubstituted pyrazoline derivatives bearing 2-hydroxy and 4-methoxy group at aromatic ring attached to C-3 of pyrazoline ring as antioxidant agents. The substitution pattern of the pyrazoline ring was rationalized so as to be correlated to the diaryl heterocycles template of compounds that are known to act selectively as COX-2 inhibitors.\textsuperscript{24}

### 3.2. Synthetic discussion of pyrazolines (5)

The target compounds 1-(4-aminosulfonylphenyl)-3,5-diarylpyrazolines (5a-m) were synthesized by following the synthetic route outlined in Scheme 3.1. The intermediate chalcones 3 were synthesized by base catalyzed Claisen-Schmidt condensation reaction of the appropriate acetophenones 1 with substituted benzaldehydes 2.\textsuperscript{22,25} The target 1-(4-aminosulfonylphenyl)-3,5-diarylpyrazolines (5a-n) were obtained by condensation of appropriate chalcones 3 and 4-hydrazinobenzenesulfonamide hydrochloride (4) in refluxing acidic ethanol. 4-Hydrazinobenzenesulfonamide hydrochloride (4) in turn was prepared via diazotization of sulfanilamide followed by reduction of the corresponding diazonium salt with stannous chloride.\textsuperscript{26} The synthetic details of each step are given in the following text.
Synthesis of Pyrazolines as Antimicrobials, Antioxidants and Anti-inflammatory agents

Scheme 3.1. Synthesis of pyrazolines 5. (i) KOH/MeOH, stir; (ii) EtOH/AcOH, reflux.

Scheme 3.2. Mechanism of chalcone 3 formation from appropriate acetophenone 1

Novel chalcones 3c, 3e and 3j prepared during this study have been reported by us\textsuperscript{22} for the first time. The structures of chalcones were confirmed by their spectral
data. All the synthesized chalcones in the present study were found to be geometrically pure with trans-configuration as indicated by the coupling constant ($J_{\text{H}^\alpha-\text{H}^\beta} = 16.2-15.3$ Hz) in their $^1$H NMR spectra. In general two trans protons corresponding to chalcone moiety appeared as two doublets. The proton at $\beta$-position to the carbonyl group resonates more downfield at $\delta 8.12-7.74$ with coupling constant 16.2-15.3 Hz while the $\alpha$-proton resonates at $\delta 7.62-7.35$. In addition to characteristic trans protons, signals corresponding to methoxy group in all cases and hydroxy group in the case of 3k, 3l, 3m and 3n also appeared as expected in their $^1$H NMR spectra.

3.2.2. Synthesis of pyrazolines (5)

The target 1-(4-aminosulfonylphenyl)-3,5-diarylpyrazolines (5) have been synthesized by condensing the appropriately substituted chalcones 3 with 4-hydrazinobenzenesulfonyamide hydrochloride (4) in ethanol in acidic medium. The mechanism of the reaction is depicted in Scheme 3.3. The mechanism involves an initial attack of nucleophilic terminal nitrogen of hydrazine on the carbonyl carbon to form an intermediate 9 that undergoes second nucleophilic attack of imine nitrogen resulting in cyclization yielding 10, which on rearrangement afforded pyrazoline ring 5.

![Scheme 3.3. Mechanism of pyrazolines 5 formation from chalcones 3](image)

The structure of newly synthesized 1-(4-aminosulfonylphenyl)-3,5-diarylpyrazolines (5) were characterized by their spectral ($^1$H NMR, $^{13}$C NMR, IR, and Mass) and elemental analysis data. The IR spectrum of pyrazoline 5a exhibited two absorption bands at 3315 cm$^{-1}$ & 3235 cm$^{-1}$ which could be assigned to N-H stretching. The functional group region showed strong absorption bands at 1595 cm$^{-1}$ for C≡N stretching and two bands at 1322 cm$^{-1}$ & 1151 cm$^{-1}$ for SO$_2$ stretching. $^1$H NMR spectrum of 5a showed characteristic ABX pattern of three protons of pyrazoline including two at C4 and one at C5. C$_3$-H of pyrazoline resonates at $\delta 5.72$ as a doublet of doublet with coupling constants of 4.8 Hz and 12.3 Hz. The cis C$_4$-H appeared as a doublet of doublet at $\delta 3.97$ with coupling constants 17.4 Hz and 12.6...
Hz. The trans C₄-H also appeared as a doublet of doublet at δ 3.16 with coupling constants 17.1 Hz and 4.8 Hz. The two singlets at δ 3.84 and 3.81 could be attributed to the two methoxy groups present on the aromatic ring attached to C5 of the pyrazoline ring. A singlet exchangeable in D₂O, present at δ 7.04 could be assigned to the SO₂NH₂ group present at 4-position of phenyl ring attached to N-1 of pyrazoline ring. The other protons as expected for the proposed structure also appeared in the aromatic region. The ¹³C NMR spectrum of 5a exhibited four doublets at δ 163.1 (d, ¹JC₅F = 247.6 Hz), δ 116.1 (d, ²JC₅F = 21.7 Hz), δ 128.6 (d, ³JC₅F = 8.3 Hz) and δ 128.9 (d, ⁴JC₅F = 3.0 Hz) which confirmed the presence of fluorine atom at para position of the aromatic ring attached to C3 of pyrazoline ring. Surprisingly four bond C-F coupling was observed in 5a which was not commonly seen in other analogues. Two large signals at δ 60.7 and δ 56.1 could be attributed to the two methoxy groups present on the ring attached to C5 of pyrazoline ring. Chemical shift values of δ 58.4 and 42.3 due to C₅- and C₄-pyrazoline carbon atoms further confirmed the pyrazoline structure. IR spectra of 5b-5n were quite similar to that of 5a. ¹H NMR spectra of 5b-5n also exhibited characteristic signals due to ABX type pattern of three pyrazoline protons, sulfonamide group and methoxy groups as well as other aromatic protons at appropriate δ values similar to that of 5a. ¹³C NMR spectra of 5c & 5d displayed four doublets while compound 5b & 5e displayed three doublets (four bond coupling not observed) confirming the presence of fluorine at para position of aromatic ring attached to C3 of pyrazoline ring while signals due to methoxy groups also appeared at appropriate δ values.

¹H NMR spectra of 5k-5m exhibited other characteristic signal due to hydroxy substituent present at ortho position of the aromatic ring attached to C3 of pyrazoline ring around δ 10.53-10.47 while singlet due to methoxy group present at para position of aromatic ring attached to C3 of pyrazoline ring appeared in the range of δ 3.82-3.73 along with two other methoxy groups present on aromatic ring attached to C5 of pyrazoline ring.

3.3. Biological testing results

Thirteen of the fourteen newly synthesized 1-(4-aminosulfonylphenyl)-3,5-diarylpyrazolines (5a-m) were screened for their in vivo anti-inflammatory (AI)
activity by carrageenan-induced rat paw edema assay in male Wistar albino rats. The protocol of animal experiments has been approved by the Institutional Animal Ethics Committee (IAEC). Each test compound was dosed orally (50 mg/kg body weight) 30 minutes prior to induction of inflammation by carrageenan injection. Indomethacin was used as a reference AI drug at a dose of 10 mg/kg intraperitoneally. The AI activity was then calculated 1-4 h after carrageenan injection and presented in Table 3.1 as the mean paw volume (mL) in addition to the percentage AI activity (AI%). All the newly synthesized compounds were also evaluated for their in vitro antibacterial activity against two Gram-positive and two Gram-negative bacteria as well as for their antifungal activity against two fungi with a view to find new leads for a monotherapy of an AI drug with antimicrobial properties. Additionally, four of the newly synthesized 1-(4-aminosulfonylphenyl)-3,5-diarylpyrazolines (5k-5n) bearing phenolic substituents were evaluated as potential antioxidant agents using four different methods. The AI testing using Wistar rats was performed in collaboration with the Institute of Pharmaceutical Science whereas antibacterial evaluation was carried out in the laboratories of Department of Microbiology at our University. The antioxidant testing was performed in the laboratories of Prof. Luciano Saso, at Department of Physiology and Pharmacology "Vittorio Erspamer", Sapienza University of Rome, Rome, Italy.

### 3.3.1. Anti-inflammatory activity

Determination of the inhibition of swelling induced in rat paw edema is one of the most popular methods used for testing AI activity of newly synthesized compounds. In this method a small amount of solution or suspension of a phlogistic agent (edemogen) is injected into the planter tissues of the hind paw of a rat and the amount of swelling is measured by determining the paw volume using a plethysmometer (model 7140, Ugo Basile, Italy). The most widely used assay in this category is carrageenan-induced edema in rats introduced by Winter et al. in 1962.\(^\text{30}\)
3.3.1.1. Pharmacological assay

The detailed description of the procedure followed is given in chapter 2. The edema was expressed as an increase in the volume of paw, and the percentage of edema inhibition for each rat and each group was obtained as:

\[
\% \text{ inhibition} = \left( \frac{(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{test}}}{(V_t - V_0)_{\text{control}}} \right) \times 100
\]

Where \( V_t \) = volume of edema at specific time interval and \( V_0 \) = volume of edema at zero time interval.

3.3.1.2. Anti-inflammatory activity – Results and discussion

Increase in the volume of paw and the percentage of edema inhibition for each rat group for each compound is summarized in Table 3.1. Considering the fact that carrageenan induced paw edema assay is a biphasic event involving the release of histamine and serotonin as the mediators of inflammation in the first phase which normally lasts for about 2h after the carrageenan injection, AI activity up to 2h after carrageenan injection can be attributed to the anti-histamine and anti-serotonin activity of the synthesized compounds. The second phase which generally operates between 2h-4h after the carrageenan injection involves prostaglandins as the mediators for the inflammation. Thus any AI activity in this period can be attributed to the inhibition of prostaglandin synthesis. A careful study of the results shown in Table 3.1 revealed that compound 5i showed potent anti-inflammatory activity (68.3%) comparable with that of indomethacin (77.2%), whereas compounds 5a, 5d, 5g, 5h & 5m displayed good anti-inflammatory activity (62.8-53.8%) 3h after the carrageenan injection. Good AI activity in the second phase of biphasic carrageenan edema assay indicates the capability of the tested compounds to inhibit prostaglandin synthesis. This can be attributed to their ability to bind cyclooxygenase (COX), an enzyme responsible for the synthesis of prostaglandins.

Table 3.1. In vivo anti-inflammatory (AI) activity of compounds 5a-5m in carrageenan-induced rat paw edema assay (acute inflammatory model)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Volume of edema (mL) and % AI</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>Control</td>
<td>0.69 ± 0.12</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.21 ± 0.06** (69.6)</td>
</tr>
<tr>
<td></td>
<td>5a</td>
</tr>
<tr>
<td>---</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>0.28 ± 0.05**</td>
</tr>
<tr>
<td></td>
<td>(59.4)</td>
</tr>
</tbody>
</table>

*a*Significantly different compared to respective control values, *P* < 0.05.

**Significantly different compared to respective control values, *P* < 0.01.

a Dose level: test compounds (50 mg/kg body wt), indomethacin (10 mg/kg body wt).

b Values are expressed as mean ± SEM (number of animals = 6) and analyzed by ANOVA.

c Values in parentheses (percentage anti-inflammatory activity, AI%).

Although from the available data, no clear correlation can be drawn between the positional preference of substituents and AI activity, the compounds having methoxy substituents at 3,4-position of the aromatic ring attached to C5 of the pyrazoline ring exhibits more anti-inflammatory activity compared to other substitutions.

### 3.3.2. Antimicrobial assay

Thirteen of the fourteen synthesized 1-(4-aminosulfonylphenyl)-3,5-diarylpyrazolines have been evaluated for their *in vitro* antibacterial activity against
two Gram-positive and two Gram-negative bacteria. In addition to this, these compounds were also evaluated for their in vitro antifungal activity against two fungi. All the microbial cultures used in the present study were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH) Chandigarh, India.

### 3.3.2.1. Test microorganism

Four bacterial and two fungal strains were selected on the basis of their clinical importance in causing diseases in humans. *Staphylococcus aureus* (*S. aureus*) (MTCC 96), *Bacillus subtilis* (*B. subtilis*) (MTCC 121) representing Gram-positive bacteria, *Escherichia coli* (*E. coli*) (MTCC 1652) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (MTCC 741) representing Gram-negative bacteria, and two yeasts, *Candida albicans* (*C. albicans*) (MTCC 227), and *Saccharomyces cerevisiae* (*S. cerevisiae*) (MTCC 170) were used for evaluation of antimicrobial activity of the compounds. The bacteria were sub cultured on Nutrient agar whereas yeast on Malt yeast agar.

### 3.3.2.2. Antimicrobial assay

The antimicrobial activity of thirteen newly synthesized compounds 5a-5m was evaluated by the agar well diffusion method.\(^{32,33}\) The detailed description of procedure followed is given in chapter 2.

### 3.3.2.3. Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of various compounds against bacterial strains was tested through a modified agar well diffusion method\(^{34}\) described in detailed in chapter 2.

### 3.3.2.4. Antimicrobial activity – Results and discussion

Most of the tested chemical compounds possessed moderate antibacterial activity against both the Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) bacteria (Table 3.2). However, all the tested compounds were found to be inactive against Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Amongst the tested compounds, 5k exhibited the lowest MIC of 32 µg/mL against *B. subtilis* whereas 5g
showed MIC of 64 µg/mL against *S. aureus* and *B. subtilis*. Though the tested compounds failed to show any appreciable activity against the tested bacterium, the results against two fungal strains were encouraging. Six (*5a, 5f, 5g, 5h, 5j* and *5k*) out of thirteen tested compounds (*5a-5m*) showed antifungal activity with MIC in the range of 32-64 µg/mL that is better than the reference drug amphotericin-B which showed the MIC of 100 µg/mL against *S. cerevisiae*. However, only one of the tested compounds *5f* was found to be better than the reference drug amphotericin-B against *Candida albicans*. Four compounds (*5a, 5g, 5h* and *5k*) showed antifungal activity comparable to the standard drug against *Candida albicans*. Though no clear correlation between the position of the substituents and antifungal activity could be drawn, compounds having an *ortho* methoxy group in the C5-phenyl were found to be exhibiting better antifungal activity.

### Table 3.2. *In vitro* antimicrobial activity of *5a-5m* using agar well diffusion method

<table>
<thead>
<tr>
<th>Compound</th>
<th>Diameter of zone of inhibition in mm&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>S. aureus</em></th>
<th><em>B. subtilis</em></th>
<th><em>S. cerevisiae</em></th>
<th><em>C. albicans</em></th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>5a</em></td>
<td>17.6 (128)</td>
<td>18.3 (64)</td>
<td>15.6 (64)</td>
<td>13.6 (128)</td>
<td></td>
</tr>
<tr>
<td><em>5b</em></td>
<td>15.3 (128)</td>
<td>16.3 (128)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>5c</em></td>
<td>13.6 (256)</td>
<td>15.0 (128)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>5d</em></td>
<td>14.0 (256)</td>
<td>15.3 (128)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>5e</em></td>
<td>12.6 (&gt;256)</td>
<td>13.6 (256)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>5f</em></td>
<td>13.6 (256)</td>
<td>15.0 (128)</td>
<td>17.3 (32)</td>
<td>15.3 (64)</td>
<td></td>
</tr>
<tr>
<td><em>5g</em></td>
<td>18.3 (64)</td>
<td>19.3 (64)</td>
<td>16.0 (64)</td>
<td>13.6 (128)</td>
<td></td>
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<tr>
<td><em>5h</em></td>
<td>15.3 (128)</td>
<td>18.6 (64)</td>
<td>17.6 (32)</td>
<td>14.3 (128)</td>
<td></td>
</tr>
<tr>
<td><em>5i</em></td>
<td>13.6 (256)</td>
<td>17.3 (128)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>5j</em></td>
<td>12.0 (&gt;256)</td>
<td>13.6 (&gt;256)</td>
<td>16.3 (64)</td>
<td>12.6 (&gt;256)</td>
<td></td>
</tr>
<tr>
<td><em>5k</em></td>
<td>18.6 (64)</td>
<td>20.6 (32)</td>
<td>15.6 (64)</td>
<td>14.3 (128)</td>
<td></td>
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<tr>
<td><em>5l</em></td>
<td>15.6 (128)</td>
<td>18.6 (64)</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td><em>5m</em></td>
<td>13.3 (256)</td>
<td>14.3 (256)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>26.6 (5)</td>
<td>24.0 (5)</td>
<td>Nt</td>
<td>Nt</td>
<td></td>
</tr>
<tr>
<td>Amphotericin-B</td>
<td>Nt</td>
<td>Nt</td>
<td>13.6 (100)</td>
<td>14.3 (100)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Values, including diameter of the well (8mm), are means of three replicates. Values in parentheses; MIC (µg/mL) = minimum inhibitory concentration; Nt = Not tested; - No activity.
3.3.3. Antioxidant testing

In the present investigation four pyrazolines bearing free hydroxy group at ortho position of aromatic ring attached to C3 of pyrazoline ring were selected and tested for their antioxidant activity in the labs of Prof. Luciano Saso, at Department of Physiology and Pharmacology "Vittorio Erspamer", Sapienza University of Rome, Rome, Italy. The four pyrazolines tested in the present study are 5k, 5l, 5m and 5n.

3.3.3.1. Material used

2,2'-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazine-6-sulfonic acid) (ABTS), sulfanilamide, linoleic acid (LA), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride-6H2O, sodium acetate, potassium persulphate, were purchased from Sigma-Aldrich or other standard suppliers.

Thin layer chromatography (TLC) analyses were performed on aluminium silica gel sheets 60 F254 plates (Merck, Darmstadt, Germany) and spots were detected using a UV lamp at 254 nm.

3.3.3.2. Measurement of antioxidant activity

3.3.3.2.1. DPPH radical scavenging activity

Scavenging activity of compounds against DPPH radical was assessed according to the method of Blois with some modifications.\(^\text{35}\) Briefly, 2 ml of each compound in EtOAc (1 mM) was mixed with 2 ml of DPPH methanol solution (0.1 mM). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Ascorbic acid (1 mM) and BHT (1 mM) were used as references. The ability to scavenge DPPH radical was calculated by the following equation:

\[
\text{DPPH radical scavenging activity, (\%)} = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100
\]

where \(\text{Abs}_{\text{control}}\) is the absorbance of DPPH radical in EtOAc (ethyl acetate), \(\text{Abs}_{\text{sample}}\) is the absorbance of DPPH radical solution mixed with sample. All determinations were performed in triplicate (\(n=3\)).
3.3.3.2.2. ABTS•+ radical scavenging activity

For ABTS•+ assay, the procedure followed the method of Arnao et al.\textsuperscript{36} with some modifications. The stock solutions included 7 mM ABTS•+ solution and 2.4 mM potassium persulphate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 14 h at room temperature in the dark. The solution was then diluted by mixing 2 ml ABTS•+ solution with 30 ml methanol to obtain an absorbance of 0.706 ± 0.01 units at 734 nm using a spectrophotometer. A fresh ABTS•+ solution was prepared for each assay. 0.1 ml of compound in EtOAc (1 mM) was allowed to react with 2 ml of the ABTS•+ solution and the absorbance was taken at 734 nm after 2 min. The ABTS•+ scavenging capacity of the compound was compared with that of BHT and ascorbic acid and percentage inhibition calculated as:

\[
\text{ABTS•+ radical scavenging activity, } \% = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100
\]

where \(\text{Abs}_{\text{control}}\) is the absorbance of ABTS•+ radical in methanol; \(\text{Abs}_{\text{sample}}\) is the absorbance of an ABTS•+ radical solution mixed with sample. All determinations were performed in triplicate (n=3).

3.3.3.2.3. Ferric reducing/antioxidant power (FRAP)

The FRAP assay was done according to the method described by Benzie and Strain\textsuperscript{37} with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g C\textsubscript{2}H\textsubscript{3}NaO\textsubscript{2}×3H\textsubscript{2}O and 16 ml C\textsubscript{2}H\textsubscript{4}O\textsubscript{2}), pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM FeCl\textsubscript{3}×6H\textsubscript{2}O solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml FeCl\textsubscript{3}×6H\textsubscript{2}O solution and then warmed at 37 °C before using. 0.15 ml of compound in EtOAc (1 mM) was allowed to react with 2.8 ml of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyl triazine complex) were then taken at 593 nm. Results are expressed in mM Trolox equivalent (TE). Ascorbic acid and BHT was used as references. All determinations were performed in triplicate (n=3).

3.3.3.2.4. Determination by the ferric thiocyanate (FTC) method

The antioxidant activity of compounds against lipid peroxidation was measured through ammonium thiocyanate assay, as described by Takao \textit{et al.}\textsuperscript{38} with some modifications. The reaction solution, containing 0.2 ml of 1 mM compound in
EtOAc, 0.2 mL of linoleic acid emulsion (25 mg/ml in 99% ethanol) and 0.4 ml of 50 mM phosphate buffer (pH 7.4), was incubated in the dark at 40 °C. A 0.1 mL aliquot of the reaction solution was then added to 3 ml of 70% (v/v) ethanol and 0.05 ml of 30% (w/v) ammonium thiocyanate. Precisely 3 min after the addition of 0.05 ml of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid to the reaction mixture, the absorbance of the resulting red color was measured at 500 nm. Aliquots were assayed every 24 h until the day after the absorbance of the control solution (without compound) reached maximum value. BHT and Ascorbic acid were used as references. All determinations were performed in triplicate (n=3).

3.3.3.3. Antioxidant activity – Results and discussion

It is widely accepted that to characterize the properties of antioxidant agents, different validated benchmark methods are needed. One has to point out that the antioxidant activity measured by an individual assay reflects only the chemical reactivity under the specific conditions applied in that assay. For example, in our study we used three different simple redox-based assays (all involving one redox reaction with the oxidant) for measuring the reducing capacity of the test compounds. Among these assays, the DPPH assay is influenced by the kinetic behaviour of the antioxidant compound, while the FRAP assay and the ABTS assay are carried out at acidic and neutral conditions, respectively. Other assay employed in this study has been selected to evaluate the antioxidant activity of the pyrazolines under investigation in linoleic acid system by ferric thiocyanate (FTC) method. The radical scavenging and total antioxidant activity of compounds (1 mM) were compared with those of BHT and ascorbic acid at the same concentration and expressed as % of inhibition against DPPH, ABTS and mM TE, respectively (Table 3.3).

<table>
<thead>
<tr>
<th>Compound (1 mM)</th>
<th>DPPH %</th>
<th>ABTS %</th>
<th>FRAP mM TE</th>
</tr>
</thead>
<tbody>
<tr>
<td>5k</td>
<td>57.7 ± 1.0</td>
<td>80.3 ± 0.8</td>
<td>-</td>
</tr>
<tr>
<td>5l</td>
<td>58.5 ± 0.4</td>
<td>80.4 ± 0.6</td>
<td>-</td>
</tr>
<tr>
<td>5m</td>
<td>58.4 ± 0.1</td>
<td>80.6 ± 0.5</td>
<td>-</td>
</tr>
<tr>
<td>5n</td>
<td>57.2 ± 0.4</td>
<td>80.7 ± 0.3</td>
<td>-</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>97.4 ± 0.2</td>
<td>&gt; 100</td>
<td>311.6 ± 0.4</td>
</tr>
<tr>
<td>BHT</td>
<td>77.4 ± 0.6</td>
<td>15.3 ± 0.7</td>
<td>123.1 ± 0.9</td>
</tr>
</tbody>
</table>
DPPH and ABTS assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation end-point. ABTS or DPPH radical scavenging methods are common spectrophotometer procedures for determining the antioxidant capacities of components. All substances demonstrated moderate DPPH activity compared to the controls. The scavenging effect of the samples decreased in the order: Ascorbic acid (97.4 % ± 0.2 %) > BHT (77.4 % ± 0.6 %) > 5l (58.5 % ± 0.4 %) ≈ 5m (58.4 % ± 0.1 %) ≈ 5k (57.7 % ± 1.0 %) ≈ 5n (57.2 % ± 0.4 %). The ABTS activity of all compounds was stronger than BHT and ranged from 80.3 % ± 0.8 % (5k) to 80.7 % ± 0.3 % (5n). There were no significant differences between radical scavenging activities of the four pyrazolines.

In FRAP assay reduction of ferric tripyridyl triazine complex to ferrous form (intense blue color) at low pH can be monitored by measuring the change in absorption at 593 nm. The change in absorbance is therefore, directly related to the combined or “total” reducing power of the electron donating antioxidants present in the reaction mixture. The tested substances did not manifest any FRAP activity probably because of the impossibility to the breaking the free radical chain by donating a hydrogen atom.

During linoleic acid peroxidation, peroxides were formed and these compounds oxidized Fe²⁺ to Fe³⁺. The Fe³⁺ ion formed a complex with SCN⁻, which had a maximum absorbance at 500 nm (Table 3.4.). Thus, a high absorbance value was an indication of high peroxide formation. This method measures the amount of

<table>
<thead>
<tr>
<th>Compound (1 mM)</th>
<th>Absorbance at 500 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; day</td>
</tr>
<tr>
<td>Control</td>
<td>1.34 ± 0.035</td>
</tr>
<tr>
<td>5k</td>
<td>1.34 ± 0.010</td>
</tr>
<tr>
<td>5l</td>
<td>1.34 ± 0.005</td>
</tr>
<tr>
<td>5m</td>
<td>1.33 ± 0.010</td>
</tr>
<tr>
<td>5n</td>
<td>1.31 ± 0.010</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1.32 ± 0.018</td>
</tr>
<tr>
<td>BHT</td>
<td>1.34 ± 0.010</td>
</tr>
</tbody>
</table>
peroxide produced during the initial stages of oxidation, which is the primary product of oxidation. As shown in Figure 3.1 the absorbance of the control at 500 nm increased to a maximal value of 4 after 72 h. All determined pyrazolines hindered the oxidation of linoleic acid for all five days. The highest significant diminution was demonstrated by 5m (1.65 ± 0.009) followed by 5l (1.85 ± 0.048). However, the antioxidant activity of these substances was slightly less effective than that of BHT, a widely used commercial antioxidant. Ascorbic acid demonstrated pro-oxidative effect at this concentration.

3.4. Conclusions
The objective of the synthesis and biological evaluation of a new series of 1-(4-aminosulfonylphenyl)-3,5-diarylp yrazolines (5) was to find the dual anti-inflammatory-antimicrobial agents. Amongst the tested compound 5i showed pronounced anti-inflammatory activity (68.3% inhibition after 3h) and some of the compounds, 5d, 5g, 5h, & 5m exhibited good anti-inflammatory activity ≥ 55%. Though the tested compounds failed to give encouraging results in terms of their
antibacterial properties, the antifungal activity results are promising and demand further investigations in this area. For instance, six (5a, 5f, 5g, 5h, 5j and 5k) out of thirteen tested compounds (5a-m) showed better antifungal activity than the reference drug amphotericin-B against yeast *S. cerevisiae*.

In this study, four 1,3,5-triarylsubstituted pyrazoline derivatives having *ortho* hydroxyl group in the phenyl ring attached to C3 of pyrazoline ring were selected for their *in vitro* evaluation as antioxidants by different antioxidant assays (DPPH, ABTS+, and FRAP assays), and by ferric thiocyanate method. The results obtained in this study suggest that pyrazolines containing a phenolic moiety may be considered as a possible scaffold for searching new antioxidant compounds. In general the activity was not found to be significantly affected by the position of methoxy groups in the phenyl moiety at C5 of the pyrazoline. The ABTS activity of all the compounds was stronger than BHT while a moderate DPPH activity was reported for all the compounds. The tested compounds did not manifest any FRAP activity while they hindered the oxidation of linoleic acid for all five days.
3.5. Experimental Section

Melting points were determined in open glass capillaries in an electrical melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu-21 FT-IR or Perkin-Elmer IR Spectrophotometer or ABB MB 3000 DTGS IR instrument using the KBr pellet technique. $^1$H NMR and $^{13}$C NMR spectra were recorded either in pure DMSO-$d_6$ or in CDCl$_3$ on Bruker NMR spectrometers at 300 MHz and 75.5 MHz respectively using tetramethylsilane (TMS) as internal standard. Chemical shifts are expressed in $\delta$, ppm. $^1$H NMR data are reported in order: multiplicity (br, broad; s, singlet; d, doublet; t, triplet; m, multiplet; ex, exchangeable by D$_2$O), approximate coupling constant in Hertz, number of protons and type of protons. The purity of the compounds was checked by $^1$H NMR and thin layer chromatography (TLC) on silica gel plates using a mixture of petroleum ether and ethyl acetate as eluent. Iodine or UV lamp was used as a visualizing agent. Mass spectra were recorded on JEOL-AccuTOF JMS-T100LC Mass spectrometer having DART (Direct Analysis in Real Time) source in ES$^+$ mode.

3.5.1. Synthesis of 4-hydrazinobenzenesulfonamide hydrochloride (4)

A cold, stirred suspension of sulfanilamide (1 mol eq) in hydrochloric acid (60 mL) was diazotized by the drop wise addition of sodium nitrite (1.2 mol eq) in water (12.5 mL) over 30 minutes. To the cold diazonium salt solution thus formed was added a cooled suspension of stannous chloride (2 mol eq) in hydrochloric acid (75 mL) with vigorous stirring, and the resulting mixture was stirred for 4 h. The precipitated 4-hydrazinobenzenesulfonamide hydrochloride (4) was filtered, dried and crystallized from ethanol. m.p. 218-219 °C, Lit.$^{26}$ m.p. 225 °C, yield 55.6%.

3.5.2. Representative procedure for synthesis of chalcones (3)

To a stirred methanolic solution of NaOH (10 mmol) and appropriate benzaldehyde 2 (1 mol eq) at ice cold condition was added appropriate acetophenone 1 (1 mol eq) over 15 minute. The reaction mixture was stirred for 18 hour at ambient temperature. The course of the reaction was monitored with TLC using ethyl acetate/petroleum ether as eluent. The contents of the flask were poured into crushed ice and neutralization with dil. HCl resulted in precipitation of the yellowish solid. The solid so obtained was filtered, washed with water and dried. The crude was
crystallized from ethanol-chloroform (8:2) to afford the target chalcones 3 as crystalline compounds in excellent yield.

(E)-1-(4-fluorophenyl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one (3a)
m.p. 68-69 °C (Lit.\textsuperscript{40} 69 °C), yield 88%; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): \(\delta\) 8.12 (d, \(J = 15.9\) Hz, 1H, COCH=CH\textsubscript{2}), 8.09-8.05 (m, 2H, Ar-H), 7.59 (d, \(J = 15.9\) Hz, 1H, COCH=CH\textsubscript{2}), 7.19 (t, \(J = 8.7\) Hz, 2H, Ar-H), 7.12 (t, \(J = 8.1\) Hz, 2H, Ar-H), 6.99 (d, \(J = 8.1\) Hz, 1H, Ar-H), 3.91 (s, 3H, OCH\textsubscript{3}), 3.90 (s, 3H, OCH\textsubscript{3}).

(E)-1-(4-fluorophenyl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (3b)
m.p. 134-135 °C (Lit.\textsuperscript{41} 136 °C), yield 83%; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): \(\delta\) 8.08 (d, \(J = 15.6\) Hz, 1H, COCH=CH\textsubscript{2}), 8.07-8.03 (m, 2H, Ar-H), 7.56 (d, \(J = 15.6\) Hz, 1H, COCH=CH\textsubscript{2}), 7.21-7.16 (m, 3H, Ar-H), 6.95 (dd, \(J = 2.4, 9.0\) Hz, 1H, Ar-H), 6.90 (d, \(J = 9.0\) Hz, 1H, Ar-H), 3.89 (s, 3H, OCH\textsubscript{3}), 3.84 (s, 3H, OCH\textsubscript{3}).

(E)-1-(4-fluorophenyl)-3-(2,5-dimethoxyphenyl)prop-2-en-1-one (3c)
m.p. 110-112 °C, yield 86%; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): \(\delta\) 8.06 (t, \(J = 7.2\) Hz, 2H, Ar-H), 7.77 (d, \(J = 15.6\) Hz, 1H, COCH=CH\textsubscript{2}), 7.37 (d, \(J = 15.6\) Hz, 1H, COCH=CH\textsubscript{2}), 7.25-7.17 (m, 4H, Ar-H), 6.91 (d, \(J = 8.1\) Hz, 1H, Ar-H), 3.96 (s, 3H, OCH\textsubscript{3}), 3.94 (s, 3H, OCH\textsubscript{3}).

(E)-1-(4-fluorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (3d)
m.p. 82-83 °C (Lit.\textsuperscript{40} 82 °C), yield 79%; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): \(\delta\) 8.06 (t, \(J = 7.2\) Hz, 2H, Ar-H), 7.77 (d, \(J = 15.6\) Hz, 1H, COCH=CH\textsubscript{2}), 7.37 (d, \(J = 15.6\) Hz, 1H, COCH=CH\textsubscript{2}), 7.25-7.17 (m, 4H, Ar-H), 6.91 (d, \(J = 8.1\) Hz, 1H, Ar-H), 3.96 (s, 3H, OCH\textsubscript{3}), 3.94 (s, 3H, OCH\textsubscript{3}).

(E)-1-(4-fluorophenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one (3e)
m.p. 87-88 °C, yield 80%; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): \(\delta\) 8.06 (dd, \(J = 5.7, 8.4\) Hz, 2H, Ar-H), 7.74 (d, \(J = 15.6\) Hz, 1H, -COCH=CH\textsubscript{2}), 7.46 (d, \(J = 15.6\) Hz, 1H, COCH=CH\textsubscript{2}).
7.19 (t, $J = 8.4$ Hz, 2H, Ar-H), 6.79 (d, $J = 1.5$ Hz, 2H, Ar-H), 6.57-6.55 (m, 1H, Ar-H), 3.85 (s, 6H, 2x OCH$_3$).

**$E$-1-(4-chlorophenyl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one (3f)**
m.p. 107-108 °C (Lit.$^{42}$ 109.5 °C), yield 89%; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.09 (d, $J = 15.6$ Hz, 1H, COCH=CH), 7.98-7.92 (m, 3H, Ar-H), 7.58-7.42 (m, 2H, Ar-H), 7.51 (d, $J = 15.3$ Hz, 1H, COCH=CH), 7.46 (s, 1H, Ar-H), 6.50 (s, 1H, Ar-H), 6.50 (s, 1H, Ar-H), 3.92 (s, 3H, OCH$_3$), 3.88 (s, 3H, OCH$_3$).

**$E$-1-(4-chlorophenyl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (3g)**
m.p. 119-120 °C (Lit.$^{41}$ 120 °C), yield 92%; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.07 (d, $J = 15.6$ Hz, 1H, COCH=CH), 7.96 (d, $J = 8.1$ Hz, 2H, Ar-H), 7.58 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.51 (d, $J = 15.3$ Hz, 1H, COCH=CH), 7.46 (s, 1H, Ar-H), 6.56 (d, $J = 8.4$ Hz, 1H, Ar-H), 6.50 (s, 1H, Ar-H), 3.92 (s, 3H, OCH$_3$), 3.88 (s, 3H, OCH$_3$).

**$E$-1-(4-chlorophenyl)-3-(2,5-dimethoxyphenyl)prop-2-en-1-one (3h)**
m.p. 56-58 °C (Lit.$^{43}$ 57 °C, with decomposition), yield 86%; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.09 (d, $J = 15.9$ Hz, 1H, COCH=CH), 7.97 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.55 (d, $J = 15.9$ Hz, 1H, COCH=CH), 7.48 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.17 (s, 1H, Ar-H), 6.97 (dd, $J = 2.1$, 8.7 Hz, 1H, Ar-H), 6.90 (d, $J = 9.0$ Hz, 1H, Ar-H), 3.89 (s, 3H, OCH$_3$), 3.84 (s, 3H, OCH$_3$).

**$E$-1-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (3i)**
m.p. 108-109 °C (Lit.$^{44}$ 107-109 °C), yield 82%; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.97 (d, $J = 7.8$ Hz, 2H, Ar-H), 7.78 (d, $J = 15.6$ Hz, 1H, COCH=CH), 7.49 (d, $J = 7.8$ Hz, 2H, Ar-H), 7.35 (d, $J = 15.6$ Hz, 1H, COCH=CH), 6.25 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.17 (s, 1H, Ar-H), 6.92 (d, $J = 8.4$ Hz, 1H, Ar-H), 3.97 (s, 3H, OCH$_3$), 3.96 (s, 3H, OCH$_3$).
(E)-1-(4-chlorophenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one (3j)
m.p. 92-94 °C, yield 87%; ¹H NMR (300 MHz, CDCl₃): δ
7.98 (d, J = 8.4 Hz, 2H, Ar-H), 7.75 (d, J = 15.6 Hz, 1H,
COCH=CH), 7.45 (d, J = 15.6 Hz, 1H, COCH=CH), 7.50
(d, J = 8.4 Hz, 2H, Ar-H), 6.79 (d, J = 2.4 Hz, 2H, Ar-H), 6.56 (s, 1H, Ar-H), 3.86 (s, 6H, 2x OCH₃).

(E)-1-(2-hydroxy-4-methoxyphenyl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one (3k)
m.p. 129-130 °C (Lit. 25 130 °C), yield 91%; ¹H NMR
(300 MHz, CDCl₃): δ 13.43 (s, 1H, OH), 8.12 (d, J =
15.6 Hz, 1H, COCH=CH), 7.78 (d, J = 9.9 Hz, 1H, Ar-
H), 7.62 (d, J = 15.9 Hz, 1H, COCH=CH), 7.21 (s, 1H, Ar-H), 7.06 (t, J = 8.1 Hz, 1H, Ar-H), 6.93 (d, J = 8.4 Hz, 1H, Ar-H), 6.45-6.42 (m, 2H, Ar-H), 3.86 (s, 3H, OCH₃),
3.85 (s, 3H, OCH₃), 3.81 (s, 3H, OCH₃).

(E)-1-(2-hydroxy-4-methoxyphenyl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (3l)
m.p. 155-157 °C (Lit. 25 158 °C), yield 87%; ¹H NMR
(300 MHz, CDCl₃): δ 13.68 (s, 1H, OH), 8.11 (d, J =
15.3 Hz, 1H, COCH=CH), 7.82-7.79 (m, 1H, Ar-H),
7.59 (d, J = 15.9 Hz, 1H, COCH=CH), 7.55 (d, J = 9.0 Hz, 1H, Ar-H), 6.52 (dd, J =
2.4, 8.7 Hz, 1H, Ar-H), 6.47-6.44 (m, 3H, Ar-H), 3.90 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 3.83 (s, 3H, OCH₃).

(E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one (3m)
m.p. 148-150 °C (Lit. 25 150 °C), yield 85%; ¹H NMR
(300 MHz, CDCl₃): δ 13.39 (s, 1H, OH), 7.80 (d, 1H, J =
9.0 Hz, Ar-H), 7.77 (d, J = 15.6 Hz, 1H, COCH=CH),
7.49 (d, J = 15.6 Hz, 1H, COCH=CH), 6.75 (d, J = 2.1 Hz, 1H, Ar-H), 6.49 (dd, J =
2.1, 9.0 Hz, 1H, Ar-H), 6.45 (s, 2H, Ar-H), 3.84 (s, 3H, OCH₃), 3.82 (s, 6H, 2x OCH₃).
Synthesis of Pyrazolines as Antimicrobials, Antioxidants and Anti-inflammatory agents

\((E)\)-1-(2-hydroxy-4-methoxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (3n)
m.p. 157-158 °C (Lit.\(^{25}\) 159 °C), yield 75%; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 13.28 (s, 1H, -OH), 7.86 (d, \(J = 9.0\) Hz, 1H, Ar-H), 7.74 (d, \(J = 15.6\) Hz, 1H, COCH=CH), 7.47 (d, \(J = 15.6\) Hz, 1H, COCH=CH), 7.21 (s, 1H, Ar-H), 6.75 (d, \(J = 1.6\) Hz, 1H, Ar-H), 6.48 (dd, \(J = 1.6\) Hz, 9.0 Hz, 1H, Ar-H), 6.45-6.43 (m, 2H, Ar-H), 3.84 (s, 3H, OCH\(_3\)), 3.82 (s, 6H, 2x OCH\(_3\)).

3.5.3. General procedure for the synthesis of 1-(4-aminosulfonylphenyl)-3,5-diarylpyrazolines (5)

Ethanolic solution of chalcone 3, (1.00 mmol) and 4-hydrazinobenzenesulfonamide hydrochloride (4), (1.10 mmol) in presence of catalytic amount of glacial acetic acid was refluxed for 8-12 h. The course of the reaction was monitored by TLC. The contents of reaction flask were concentrated and left overnight resulting in the formation of crystals. The solid so obtained was filtered, dried over vacuum pump and crystallized from ethanol to afford the target pyrazolines 5 as fluffy colorless crystals in excellent yield.

1-(4-aminosulfonylphenyl)-3-(4-fluorophenyl)-5-(2,3-dimethoxyphenyl)pyrazoline (5a)
m.p. 220-222 °C, yield 82%; IR (cm\(^{-1}\)): 3315 & 3235 (b, N-H), 1595 (m, C=N), 1322 & 1151 (s, SO\(_2\)NH\(_2\)); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 7.84 (t, \(J = 6.3\) Hz, 2H, Ar-H), 7.60 (d, \(J = 8.1\) Hz, 2H, Ar-H), 7.29 (t, \(J = 8.1\) Hz, 2H, Ar-H), 7.04-6.96 (m, 5H, Ar-H), 6.58 (s, 1H, Ar-H), 5.72 (dd, \(J = 4.8, 12.3\) Hz, 1H, pyrazoline-H), 3.97 (dd, \(J = 12.6, 17.4\) Hz, 1H, pyrazoline-H), 3.84 (s, 3H, OCH\(_3\)), 3.81 (s, 3H, OCH\(_3\)), 3.16 (dd, \(J = 4.8, 17.1\) Hz, 1H, pyrazoline-H); \(^13\)C NMR (75.5 MHz, DMSO-\(d_6\)): \(\delta\) 163.1 (d, \(^1\)J\(_{CF}\) = 247.6 Hz), 153.1, 149.6, 146.3, 146.1, 134.9, 133.4, 128.9 (d, \(^2\)J\(_{CF}\) = 3.0 Hz), 128.6 (d, \(^3\)J\(_{CF}\) = 8.3 Hz), 127.6, 124.9, 118.3, 116.1 (d, \(^4\)J\(_{CF}\) = 21.7 Hz), 112.8, 112.1, 60.71, 58.4 (C5-pyrazoline), 56.1 (OCH\(_3\)), 42.3 (C4-pyrazoline); DART MS: \(m/z\) 456.18 ([M+H]\(^+\), C\(_{23}\)H\(_{22}\)FN\(_3\)O\(_4\)SH\(^+\) calcd. 456.13); Anal. Calcd. for C\(_{23}\)H\(_{22}\)FN\(_3\)O\(_4\)S: N, 9.23; found: N, 9.31.
**1-(4-aminosulfonylphenyl)-3-(4-fluorophenyl)-5-(2,4-dimethoxyphenyl) pyrazoline (5b)**

m.p. 178-180 °C, yield 79%; IR (cm\(^{-1}\)): 3320 & 3236 (b, N-H), 1596 (m, C=N), 1324 & 1155 (s, SO\(_2\)); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): δ 7.82 (t, \(J = 6.6\) Hz, 2H, Ar-H), 7.60 (d, \(J = 8.4\) Hz, 2H, Ar-H), 7.27 (d, \(J = 8.4\) Hz, 2H, Ar-H), 7.04 (s, 2H, SO\(_2\)NH\(_2\)), 7.00 (d, \(J = 8.4\) Hz, 2H, Ar-H), 6.77 (d, \(J = 8.1\) Hz, 1H, Ar-H), 6.64 (s, 1H, Ar-H), 6.41 (d, \(J = 8.4\) Hz, 1H, Ar-H), 5.65 (dd, \(J = 4.5, 12.0\) Hz, 1H, pyrazoline-H), 3.88 (s, 3H, OCH\(_3\)), 3.71 (s, 3H, OCH\(_3\)), 3.07 (dd, \(J = 4.5\) Hz, 17.7 Hz, 1H, pyrazoline-H); \(^{13}\)C NMR (75.5 MHz, DMSO-\(d_6\)): δ 163.5 (d, \(J_{C-F} = 246.3\) Hz), 160.5, 157.6, 149.7, 146.2, 133.2, 129.0, 128.6 (d, \(J_{C-F} = 8.3\) Hz), 127.1, 120.9, 116.1 (d, \(J_{C-F} = 21.7\) Hz), 112.1, 105.5, 99.4, 57.6 (C5-pyrazoline), 56.2 (OCH\(_3\)), 55.6 (OCH\(_3\)), 42.4 (C4-pyrazoline); DART MS: \(m/z\) 456.18 ([M+H]\(^+\), C\(_{23}\)H\(_{22}\)FN\(_3\)O\(_4\)SH\(^+\) calcd. 456.13); Anal. Calcd. for C\(_{23}\)H\(_{22}\)FN\(_3\)O\(_4\)S: N, 9.23; found: N, 9.34.

**1-(4-aminosulfonylphenyl)-3-(4-fluorophenyl)-5-(2,5-dimethoxyphenyl) pyrazoline (5c)**

m.p. 84-86 °C, yield 87%; IR (cm\(^{-1}\)): 3322 & 3240 (b, N-H), 1595 (m, C=N), 1315 & 1145 (s, SO\(_2\)); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): δ 7.86-7.81 (m, 2H, Ar-H), 7.61 (d, \(J = 8.7\) Hz, 2H, Ar-H), 7.27 (t, \(J = 8.7\) Hz, 2H, Ar-H), 7.04-7.00 (m, 5H, Ar-H, SO\(_2\)NH\(_2\)), 6.82 (dd, \(J = 2.7, 9.0\) Hz, 1H, Ar-H), 6.41 (s, 1H, Ar-H), 5.69 (dd, \(J = 4.8, 12.0\) Hz, 1H, pyrazoline-H), 3.93 (dd, \(J = 12.0, 17.4\) Hz, 1H, pyrazoline-H), 3.84 (s, 3H, OCH\(_3\)), 3.56 (s, 3H, OCH\(_3\)), 3.11 (dd, \(J = 5.1\) Hz, 17.7 Hz, 1H, pyrazoline-H); \(^{13}\)C NMR (75.5 MHz, DMSO-\(d_6\)): δ 163.1 (d, \(J_{C-F} = 245.3\) Hz), 153.7, 150.6, 149.8, 146.3, 133.2, 129.1, 128.9 (d, \(J_{C-F} = 3.0\) Hz), 128.7 (d, \(J_{C-F} = 8.3\) Hz), 116.2 (d, \(J_{C-F} = 21.7\) Hz), 113.2, 112.8, 112.2, 58.0 (C5-pyrazoline), 56.6 (OCH\(_3\)), 55.7 (OCH\(_3\)), 42.3 (C4-pyrazoline); DART MS: \(m/z\) 456.18 ([M+H]\(^+\), C\(_{23}\)H\(_{22}\)FN\(_3\)O\(_4\)SH\(^+\) calcd. 456.13); Anal. Calcd. for C\(_{23}\)H\(_{22}\)FN\(_3\)O\(_4\)S: N, 9.23; found: N, 9.28.
1-(4-aminosulfonylphenyl)-3-(4-fluorophenyl)-5-(3,4-dimethoxyphenyl) pyrazoline (5d)

m.p. 198-200 °C, yield 81%; IR (cm⁻¹): 3320 & 3235 (b, N-H), 1596 (m, C=\(\equiv\)N), 1325 & 1155 (s, SO₂); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 7.84-7.82 (m, 2H, Ar-H), 7.60 (d, \(J = 9.0\) Hz, 2H, Ar-H), 7.29 (t, \(J = 9.0\) Hz, 2H, Ar-H), 7.09 (d, \(J = 9.0\) Hz, 2H, Ar-H), 7.04 (s, 2H, SO₂NH₂), 6.94 (d, \(J = 1.8\) Hz, 1H, Ar-H), 6.88 (d, \(J = 8.4\) Hz, 1H, Ar-H), 6.68 (dd, \(J = 1.8, 8.4\) Hz, 1H, Ar-H), 5.54 (dd, \(J = 5.4, 12.0\) Hz, 1H, pyrazoline-H), 3.93 (dd, \(J = 12.0, 17.7\) Hz, 1H, pyrazoline-H), 3.72 (s, 3H, OCH₃), 3.69 (s, 3H, OCH₃), 3.19 (dd, \(J = 5.4, 17.4\) Hz, 1H, pyrazoline-H); \(^1^3\)C NMR (75.5 MHz, DMSO-\(d_6\)): \(\delta\) 163.1 (d, \(^1^3\)J\(_{C-F}\) = 246.9 Hz), 149.6, 149.3, 148.6, 146.6, 134.4, 133.5, 128.9 (d, \(^4^3\)J\(_{C-F}\) = 3.0 Hz), 128.7 (d, \(^2^3\)J\(_{C-F}\) = 8.3 Hz), 127.5, 117.9, 116.2 (d, \(^2^3\)J\(_{C-F}\) = 21.9 Hz), 112.7, 112.5, 110.3, 62.9 (C5-pyrazoline), 55.9 (OCH₃), 55.9 (OCH₃), 43.6 (C4-pyrazoline); DART MS: \(m/z\) 456.18 ([M+H]⁺, \(C_{23}H_{22}FN_{3}O_{4}S\)H⁺ calcd. 456.13); Anal. Calcd. for \(C_{23}H_{22}FN_{3}O_{4}S\): N, 9.23; found: N, 9.40.

1-(4-aminosulfonylphenyl)-3-(4-fluorophenyl)-5-(3,5-dimethoxyphenyl) pyrazoline (5e)

m.p. 210-212 °C, yield 80%; IR (cm⁻¹): 3328 & 3245 (b, N-H), 1596 (m, C=\(\equiv\)N), 1320 & 1151 (s, SO₂); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 7.84 (dd, \(J = 5.7, 8.4\) Hz, 2H, Ar-H), 7.61 (d, \(J = 9.0\) Hz, 2H, Ar-H), 7.29 (t, \(J = 9.0\) Hz, 2H, Ar-H), 7.08 (d, \(J = 9.0\) Hz, 2H, Ar-H), 7.03 (s, 2H, SO₂NH₂), 6.39 (s, 3H, Ar-H), 5.54 (dd, \(J = 5.4, 12.0\) Hz, 1H, pyrazoline-H), 3.94 (dd, \(J = 12.0, 17.7\) Hz, 1H, pyrazoline-H), 3.69 (s, 6H, 2x OCH₃), 3.20 (dd, \(J = 5.4, 17.7\) Hz, 1H, pyrazoline-H); \(^1^3\)C NMR (75.5 MHz, DMSO-\(d_6\)): \(\delta\) 161.5, 159.1 (d, \(^1^3\)J\(_{C-F}\) = 246.9 Hz), 149.5, 146.5, 144.5, 133.5, 128.8 (d, \(^2^3\)J\(_{C-F}\) = 9.1 Hz), 127.6, 116.2 (d, \(^2^3\)J\(_{C-F}\) = 21.9 Hz), 112.5, 104.2, 99.1, 63.0 (C5-pyrazoline), 55.6 (OCH₃), 43.4 (C4-pyrazoline); DART MS: \(m/z\) 456.18 ([M+H]⁺, \(C_{23}H_{22}FN_{3}O_{4}S\)H⁺ calcd. 456.13); Anal. Calcd. for \(C_{23}H_{22}FN_{3}O_{4}S\): N, 9.23; found: N, 9.38.
1-(4-aminosulfonylphenyl)-3-(4-chlorophenyl)-5-(2,3-dimethoxyphenyl)pyrazoline (5f)
m.p. 230-232 °C, yield 82%; IR (cm\(^{-1}\)): 3325 & 3240 (b, N-H), 1597 (m, C=N), 1319 & 1149 (s, SO\(_2\)); \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): δ 7.80 (d, \(J = 8.4\) Hz, 2H, Ar-H), 7.60 (d, \(J = 8.7\) Hz, 2H, Ar-H), 7.50 (d, \(J = 8.7\) Hz, 2H, Ar-H), 7.06-7.03 (m, 4H, Ar-H), 6.97 (d, \(J = 4.8\) Hz, 2H, Ar-H), 6.60-6.57 (m, 1H, Ar-H), 5.74 (dd, \(J = 5.1, 12.0\) Hz, 1H, pyrazoline-H), 3.97 (dd, \(J = 12.0, 18.0\) Hz, 1H, pyrazoline-H), 3.83 (s, 3H, OCH\(_3\)), 3.81 (s, 3H, OCH\(_3\)), 3.16 (dd, \(J = 5.4\) Hz, 18.0 Hz, 1H, pyrazoline-H); \(^{13}\)C NMR (75.5 MHz, DMSO-d\(_6\)): δ 153.2, 149.4, 146.1, 134.8, 134.1, 133.6, 131.2, 129.2, 128.2, 127.7, 124.9, 118.4, 112.9, 112.3, 60.7 (C5-pyrazoline), 58.6 (OCH\(_3\)), 49.1, 42.5 (C4-pyrazoline); DART MS: \(m/z\) 472.17/474.16 ([M+H/M+2+H]+, \(\text{C}_{23}\text{H}_{22}\text{ClN}_{3}\text{O}_{4}\text{SH}\) calcd. 472.10/474.10); Anal. Calcd. for \(\text{C}_{23}\text{H}_{22}\text{ClN}_{3}\text{O}_{4}\text{S}\): N, \(8.90\); found: N, \(8.79\).

1-(4-aminosulfonylphenyl)-3-(4-chlorophenyl)-5-(2,4-dimethoxyphenyl)pyrazoline (5g)
m.p. 208-210 °C, yield 86%; IR (cm\(^{-1}\)): 3320 & 3235 (b, N-H), 1588 (m, C=N), 1326 & 1153 (s, SO\(_2\)); \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): δ 7.75 (d, \(J = 7.8\) Hz, 2H, Ar-H), 7.69 (d, \(J = 8.4\) Hz, 2H, Ar-H), 7.43 (d, \(J = 8.1\) Hz, 2H, Ar-H), 7.01-6.98 (m, 4H, Ar-H), 6.78 (d, \(J = 8.4\) Hz, 1H, Ar-H), 6.60 (s, 1H, Ar-H), 6.38 (d, \(J = 8.4\) Hz, 1H, Ar-H), 5.64 (dd, \(J = 4.5, 11.7\) Hz, 1H, pyrazoline-H), 3.88 (s, 3H, OCH\(_3\)), 3.71 (s, 3H, OCH\(_3\)), 3.05 (dd, \(J = 4.5\) Hz, 17.7 Hz, 1H, pyrazoline-H); \(^{13}\)C NMR (75.5 MHz, DMSO-d\(_6\)): δ 160.6, 157.5, 149.2, 146.2, 134.2, 133.5, 131.3, 129.0, 127.9, 127.6, 127.1, 120.8, 112.2, 105.4, 99.3, 57.8 (C5-pyrazoline), 56.1 (OCH\(_3\)), 55.6 (OCH\(_3\)), 42.2 (C4-pyrazoline); DART MS: \(m/z\) 472.15/474.16 ([M+H/M+2+H]+, \(\text{C}_{23}\text{H}_{22}\text{ClN}_{3}\text{O}_{4}\text{SH}\) calcd. 472.10/474.10); Anal. Calcd. for \(\text{C}_{23}\text{H}_{22}\text{ClN}_{3}\text{O}_{4}\text{S}\): N, \(8.90\); found: N, \(8.96\).
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1-(4-aminosulfonylphenyl)-3-(4-chlorophenyl)-5-(2,5-dimethoxyphenyl)pyrazoline (5h)
m.p. 228-230 °C, yield 78%; IR (cm\(^{-1}\)): 3327 & 3236 (b, N-H), 1591 (m, C=N), 1325 & 1154 (s, SO\(_2\)); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 7.79 (d, \(J = 7.8\) Hz, 2H, Ar-H), 7.62 (d, \(J = 8.1\) Hz, 2H, Ar-H), 7.49 (d, \(J = 7.8\) Hz, 2H, Ar-H), 7.04-6.02 (m, 5H, Ar-H), 6.82 (d, \(J = 9.0\) Hz, 1H, Ar-H), 6.42 (s, 1H, Ar-H), 5.71 (dd, \(J = 4.5, 12.6\) Hz, 1H, pyrazoline-H), 3.92 (dd, \(J = 12.6, 17.4\) Hz, 1H, pyrazoline-H), 3.84 (s, 3H, OCH\(_3\)), 3.56 (s, 3H, OCH\(_3\)), 3.10 (dd, \(J = 4.5\) Hz, 17.7 Hz, 1H, Ar-H); \(^{13}\)C NMR (75.5 MHz, DMSO-\(d_6\)): \(\delta\) 153.7, 150.6, 149.6, 146.2, 134.2, 133.7, 131.2, 129.9, 129.2, 128.2, 127.7, 113.2, 112.8, 112.3, 58.2 (C5-pyrazoline), 56.6 (OCH\(_3\)), 55.7 (OCH\(_3\)), 42.1 (C4-pyrazoline); DART MS: \(m/\ell\) 472.15/474.16 ([M+H/2+H]+, \(\text{C_{23}H_{22}ClN}_3O_4S\) calcd. 472.17/474.16); Anal. Calcd. for \(\text{C_{23}H_{22}ClN}_3O_4S\): N, 8.90: found: N, 8.81.

1-(4-aminosulfonylphenyl)-3-(4-chlorophenyl)-5-(3,4-dimethoxyphenyl)pyrazoline (5i)
m.p. 184-186 °C, yield 85%; IR (cm\(^{-1}\)): 3328 & 3235 (b, N-H), 1595 (m, C=N), 1321 & 1151 (s, SO\(_2\)); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 7.62 (d, \(J = 7.8\) Hz, 2H, Ar-H), 7.59 (d, \(J = 6.6\) Hz, 2H, Ar-H), 7.29 (d, \(J = 8.7\) Hz, 2H, Ar-H), 7.00 (d, \(J = 8.4\) Hz, 2H, Ar-H), 6.74 (s, 2H, SO\(_2\)NH\(_2\)), 6.66 (s, 1H, Ar-H), 5.23 (dd, \(J = 6.0, 12.0\) Hz, 1H, pyrazoline-H), 3.83 (s, 3H, OCH\(_3\)), 3.77 (s, 3H, OCH\(_3\)), 3.05 (dd, \(J = 4.5\) Hz, 17.7 Hz, 1H, Ar-H); \(^{13}\)C NMR (75.5 MHz, DMSO-\(d_6\)): \(\delta\) 149.7, 148.6, 148.1, 146.8, 134.9, 133.6, 132.2, 130.6, 128.8, 127.2, 126.9, 117.8, 112.5, 111.7, 108.5, 63.6 (C5-pyrazoline), 55.9 (OCH\(_3\)), 55.8 (OCH\(_3\)), 43.5 (C4-pyrazoline); DART MS: \(m/\ell\) 472.15/474.16 ([M+H/M+2+H]+, \(\text{C_{23}H_{22}ClN}_3O_4S\) calcd. 472.10/474.10); Anal. Calcd. for \(\text{C_{23}H_{22}ClN}_3O_4S\): N, 8.90: found: N, 9.02.
1-(4-aminosulfonylphenyl)-3-(4-chlorophenyl)-5-(3,5-dimethoxyphenyl) pyrazoline (5j)

m.p. 182-184 °C, yield 76%; IR (cm⁻¹): 3326 & 3238 (b, N-H), 1589 (m, C=N), 1324 & 1143 (s, SO₂); ¹H NMR (300 MHz, DMSO-d₆): δ 7.76 (d, J = 7.8 Hz, 2H, Ar-H), 7.61 (d, J = 8.1 Hz, 2H, Ar-H), 7.44 (d, J = 7.5 Hz, 2H, Ar-H); 6.98 (s, 2H, SO₂NH₂), 6.37 (s, 3H, Ar-H), 5.76 (dd, J = 5.1, 12.0 Hz, 1H, pyrazoline-H), 4.02 (dd, J = 12.0, 17.4 Hz, 1H, pyrazoline-H); ¹³C NMR (75.5 MHz, DMSO-d₆): δ 161.5, 148.9, 146.4, 144.3, 134.3, 133.8, 131.1, 129.1, 127.9, 127.6, 112.5, 104.1, 99.2, 63.3 (C₅-pyrazoline), 55.5 (OCH₃), 43.2 (C₄-pyrazoline); DART MS: m/z 472.15/474.16 ([M+H⁺], C₂₃H₂₂ClN₃O₄S⁺ calcd. 472.10/474.10); Anal. Calcd. for C₂₃H₂₂ClN₃O₄S: N, 8.90: found: N, 8.96.

1-(4-aminosulfonylphenyl)-3-(2-hydroxy-4-methoxyphenyl)-5-(2,3-dimethoxyphenyl)pyrazoline (5k)

m. p. 218-220 °C, yield 88%; IR (cm⁻¹): 3326 & 3238 (b, N-H), 1602 (m, C=N), 1325 & 1156 (s, SO₂); ¹H NMR (300 MHz, DMSO-d₆): δ 10.53 (s, 1H, OH), 7.62 (d, J = 8.6 Hz, 2H, Ar-H); 6.98 (s, 2H, SO₂NH₂), 6.37 (s, 3H, Ar-H), 6.93 (d, J = 8.7 Hz, 2H, Ar-H), 6.65-6.52 (m, 3H, Ar-H), 5.65 (dd, J = 5.2, 12.1 Hz, 1H, pyrazoline-H), 4.06 (dd, J = 12.2, 17.7 Hz, 1H, pyrazoline-H), 3.82 (s, 6H, 2x OCH₃), 3.78 (s, 3H, OCH₃), 3.30 (dd, J = 5.2, 17.9 Hz, 1H, pyrazoline-H); ¹³C NMR (75.5 MHz, DMSO-d₆): δ 161.8, 158.2, 152.9, 152.6, 145.9, 145.5, 134.5, 133.2, 129.8, 127.6, 124.7, 118.2, 112.7, 111.6, 109.8, 106.7, 101.3, 60.4 (C₅-pyrazoline), 56.8 (OCH₃), 55.9 (OCH₃), 55.6 (OCH₃), 43.5 (C₄-pyrazoline); DART MS: m/z 484.21 ([M+H⁺], C₂₄H₂₅N₃O₆SH⁺ calcd. 484.15); Anal. Calcd. for C₂₄H₂₅N₃O₆S: N, 8.69: found: N, 8.78.
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1-(4-aminosulfonylphenyl)-3-(2-hydroxy-4-methoxyphenyl)-5-(2,4-dimethoxyphenyl)pyrazoline (5l)
m.p. 228-230 °C, yield 84%; IR (cm⁻¹): 3327 & 3243 (b, N-H), 1599 (m, C=N), 1321 & 1152 (s, SO₂); ¹H NMR (300 MHz, DMSO-d₆): δ 10.52 (s, 1H, OH), 7.61 (d, J = 8.1 Hz, 2H, Ar-H), 7.40 (d, J = 8.4 Hz, 1H, Ar-H), 7.06 (s, 2H, SO₂NH₂), 7.01-6.96 (m, 3H, Ar-H), 6.88 (d, J = 8.1 Hz, 1H, Ar-H), 6.70 (d, J = 8.1 Hz, 1H, Ar-H), 6.56-6.52 (m, 2H, Ar-H), 5.46 (dd, J = 5.4, 11.7 Hz, 1H, pyrazoline-H), 4.02 (dd, J = 12.3, 17.7 Hz, 1H, pyrazoline-H), 3.78 (s, 3H, OCH₃), 3.73 (s, 3H, OCH₃), 3.69 (s, 3H, OCH₃), 3.29 (dd, J = 5.1, 18 Hz, 1H, pyrazoline-H); ¹³C NMR (75.5 MHz, DMSO-d₆): δ 162.2, 158.5, 152.6, 149.6, 148.6, 146.2, 134.2, 133.6, 130.1, 127.7, 118.0, 112.6, 112.3, 110.3, 107.0, 101.6, 61.7 (C₅-pyrazoline), 56.0 (OCH₃), 55.8 (OCH₃), 44.7 (C₄-pyrazoline); DART MS: m/z 484.21 ([M+H]⁺, C₂₄H₂₅N₃O₆SH⁺ calcd. 484.15); Anal. Calcd. for C₂₄H₂₅N₃O₆S: N, 8.69; found: N, 8.64.

1-(4-aminosulfonylphenyl)-3-(2-hydroxy-4-methoxyphenyl)-5-(3,5-dimethoxyphenyl)pyrazoline (5m)
m.p. 232-234 °C, yield 83%; IR (cm⁻¹): 3329 & 3247 (b, N-H), 1605 (m, C=N), 1323 & 1157 (s, SO₂); ¹H NMR (300 MHz, DMSO-d₆): δ 10.47 (s, 1H, OH), 7.63 (d, J = 8.4 Hz, 2H, Ar-H), 7.41 (d, J = 8.4 Hz, 1H, Ar-H), 7.06 (s, 2H, SO₂NH₂), 6.99 (d, J = 8.7 Hz, 2H, Ar-H), 6.56 (s, 1H, Ar-H), 6.53 (d, J = 8.7 Hz, 1H, Ar-H), 6.41 (s, 3H, Ar-H), 5.46 (dd, J = 6.6, 11.7 Hz, 1H, pyrazoline-H), 4.02 (dd, J = 12.0, 17.7 Hz, 1H, pyrazoline-H), 3.78 (s, 3H, OCH₃), 3.73 (s, 6H, 2x OCH₃), 3.31 (merged with HOD peak of DMSO-d₆, 1H, pyrazoline-H); ¹³C NMR (75.5 MHz, DMSO-d₆): δ 162.2, 161.5, 158.5, 152.6, 146.1, 144.3, 133.7, 130.1, 127.7, 112.3, 110.1, 107.0, 104.3, 101.6, 99.2, 61.7 (C₅-pyrazoline), 55.8 (OCH₃), 55.6 (OCH₃), 44.6 (C₄-pyrazoline); DART MS: m/z 484.21 ([M+H]⁺, C₂₄H₂₅N₃O₆SH⁺ calcd. 484.15); Anal. Calcd. for C₂₄H₂₅N₃O₆S: N, 8.69; found: N, 8.83.
1-(4-aminosulfonylphenyl)-3-(2-hydroxy-4-methoxyphenyl)-5-(3,4-dimethoxyphenyl)pyrazoline (5n)

m.p. 208-209 °C (Lit5 210 °C), yield 75%; IR (cm⁻¹): 3325 & 3241 (b, N-H), 1598 (m, C≡N), 1321 & 1152 (s, SO₂NH₂); ¹H NMR (300 MHz, DMSO-d₆): δ 10.51 (s, 1H, OH), 7.62 (d, J = 8.1 Hz, 2H, Ar-H), 7.39 (d, J = 8.4 Hz, 1H, Ar-H), 7.05 (s, 2H, SO₂NH₂), 7.01-6.95 (m, 3H, Ar-H), 6.87 (d, J = 8.1 Hz, 1H, Ar-H), 6.70 (d, J = 8.1 Hz, 1H, Ar-H), 6.56-6.52 (m, 2H, Ar-H), 5.45 (dd, J = 6.0, 12.0 Hz, 1H, pyrazoline-H), 4.02 (dd, J = 12.0, 17.3 Hz, 1H, pyrazoline-H), 3.76 (s, 3H, OCH₃), 3.72 (s, 3H, OCH₃), 3.70 (s, 3H, OCH₃), 3.27 (dd, J = 5.6, 18 Hz, 1H, pyrazoline-H); ¹³C NMR (75.5 MHz, DMSO-d₆): δ 162.3, 158.6, 152.5, 149.7, 148.6, 146.1, 134.2, 133.6, 130.2, 127.8, 118.0, 112.6, 112.3, 110.3, 107.1, 101.6, 61.7 (C5-pyrazoline), 56.1 (OCH₃), 55.9 (OCH₃), 55.7 (OCH₃), 44.8 (C4-pyrazoline); DART MS: m/z 484.21 ([M+H]⁺, C₂₄H₂₅N₃O₆SH⁺ calcd. 484.15); Anal. Calcd. for C₂₄H₂₅N₃O₆S: N, 8.69: found: N, 8.64.
3.6. References

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